# Charged probes: Turn-on selective fluorescence for RNA

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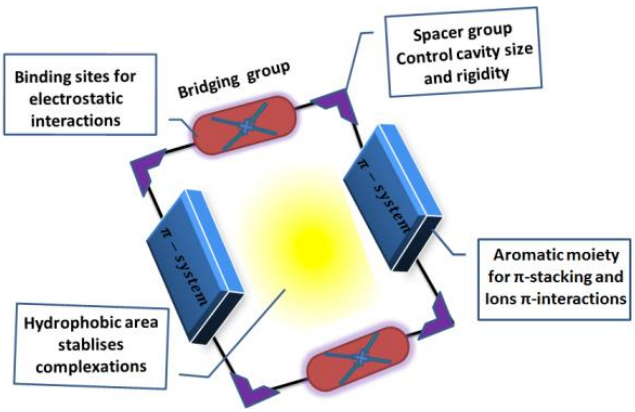
RNA controls many biological processes. The selective detection and imaging of RNA molecules can provide information about their location, kinetics, and functions at the cellular level. The imidazolium-based positively charged probes would play a significant role in the deep investigation of RNA biomolecules to check their therapeutic potential and provide aids in the future rational molecular and drug design.

## Introduction

Ribonucleic acid (RNA) plays a vital role in controlling cellular functions. It controls gene regulation and actively participates in transcriptional regulations, protein synthesis, and catalysing biological reactions.[1] Given the importance and diversity of RNA functions, charged fluorescent molecules that selectively interact with RNA may provide a novel approach of therapeutic intervention. The development and design of new, sensitive and selective probes for the detection of RNA have become a very active research field in recent years to understand the role of RNA dynamics in cellular functions. The sequencing of the genome provides the genetic overview of the proteins and nucleic acids for species but lacks the information on how these biomolecules are involved in the processes within the cells. The bio-imaging of RNA molecules can provide information about their location, kinetics, and function at cellular level.[2] Different fluorescent probes like crescent-shape,[3a] V-shape,[3b] E36 dyes,[3c] styryl,[3d-h] near-infrared,[3i] thiazole orange,[3j-m] and RuEth[3n] were studied for RNA detection. To some extent, these probes have specificity for RNA. However, to find an RNA-selective probe for living cell imaging has proven to be difficult because nucleic acid binding to small molecules generally tends to have better affinity for DNA than RNA. Considering that in the past most studies were conducted *in vitro* levels, the introduction of new type of molecules which target RNA selectively at cellular level and are used *in vivo* studies would enhance controlling cellular functions. However, The design of such synthetic molecules is correspondingly challenging, especially with good selectivity for RNA over other biomolecules.

To develop charged fluorescent probes (**CFPs**) capable of selective molecular recognition for *in vitro* and *in vivo* is one of the challenging subjects of chemical biology research.[4] Nowadays, small fluorescent probes are used excessively for monitoring cellular objectives through imaging. To develop a selective moiety for particular objective in cell biology, a number of factors play an important role such as solubility of probes in physiological media, selective and strong binding with target moiety in competitive media, selective fluorescence response, permeability through cell membrane and non-toxicity of probes for biological environment. The absence of practical fluorescent probes with high selectivity for RNA imaging *in* live cell has been a significant drawback to understand the role of RNA dynamics in cellular functions.[4a] Moreover, most of the reports for RNA sensing have limitations of poor photo-stability, high cytotoxicity, short emission wavelength and poor cell permeability. Importantly, these probes were synthesised through laborious multi-steps synthesis and time-consuming procedures.[3] Therefore, a concentrated attention should be focused towards the design and synthesise of such probes, which would create a better understanding of real-time monitoring of RNA transport in cells and spatial-temporal distribution of RNA in cellular functions. Herein, we reviewed the water soluble imidazolium based charged non-cyclic[5,6] and cyclic probes[7,8] with excellent selective fluorescent binding and imaging of RNA over other biomolecules in living cells. These charged cyclophane are easily accessible with high yield and can be applied for RNA monitoring of future potential biological applications. These stable, small charged probes are synthesised in a single step, which do not significantly disturb the RNA dynamics by involving only non-covalent binding to RNA due mainly to the electrostatic interactions and π-stacking.




**Scheme 1.**Schematic representation of binding sites in a functionalised cyclophane system

There are limited ways to address the issue of finding a proper binding way of charged fluorescent moieties with biomolecules for selective sensing which can guide to design the probes. This article embarks on finding related answers to such questions. Few examples of neutral probes are reported which gave selective turn-on fluorescence for RNA.[9] Nicholas J. Turro et al. reported pyrene binary oligonucleotide probes for selective detection of cellular mRNA through excimer emission.[9a] Yoon research group also reported a pyrene based probe for selective detection of RNA through excimer emission ''turn-on'' at physiological pH. They used their probe successfully for RNA imaging in HeLa cells.[9b] However, the **CFPs** have proven to be good choice for RNA recognition. **CFPs** consist of fluorophore units having charged hydrogen bonding with specific geometries and provide a quick response in fluorescence. Such systems are not only capable of encapsulating and stabilizing guest molecules through non-covalent interactions, but also develop additional H-π and π-π interactions with guest molecules (See scheme 1 for cyclic probes).[7,8] The water soluble systems with charged hydrogen bonding can be a good choice to develop probes for enhancing the binding affinities and selective discrimination of RNA over other biomolecules which would be highly beneficial in understanding hidden cellular processes in detail. Herein, the role of naphthalene fluorophore in imidazolium-based probes is important for developing strong interaction with RNA and producing selective turn-on fluorescence.

## RNA Sensing by Charged Probes

Imidazolium salts are formed from the protonation or substitution at nitrogen of imidazole where the positive charge is delocalized in the imidazole ring. The relatively high acidity (pKa= 21–23) of the C2 hydrogen (C2–H) of the imidazolium nucleus have made these salts to be used as effective receptors for phosphate anionic moieties of guests.[2-4]

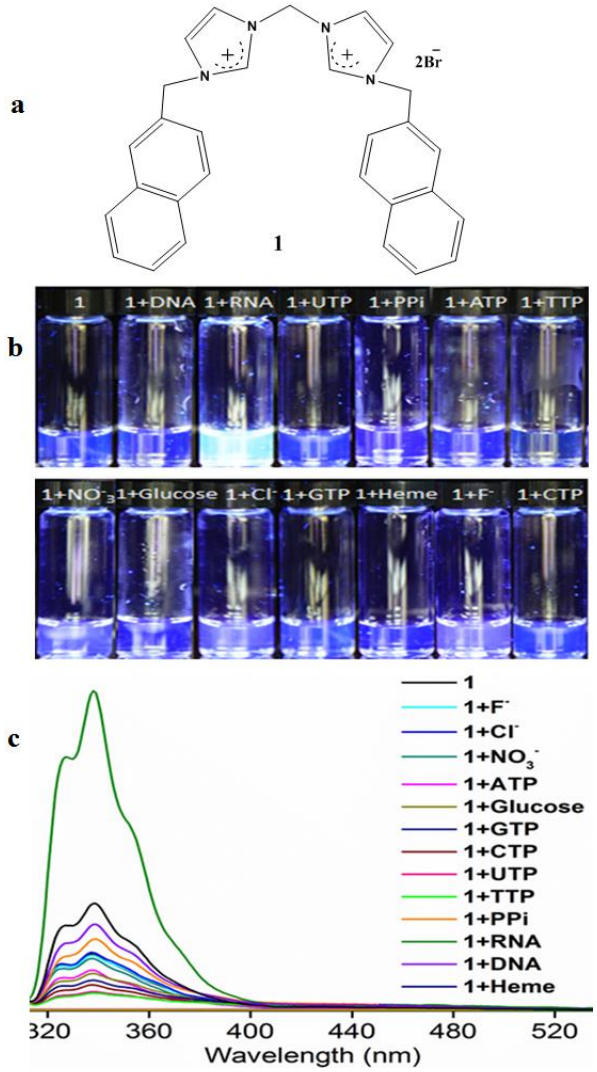



**Figure 1.** (a) Molecular structure of probe **1** (b) visual fluorescence features upon addition of guest molecules (c) Fluorescence (slit width = 5 nm; excitation at 274nm) of probe **1** (10 μM) upon addition of guest molecules (1 equiv each) at pH 7.4 (10 mM phosphate buffer). Reproduced with permission from American Chemical Society (ref. 5).

We recently reported fluorescent water soluble acyclic naphthimidazolium probe **1**, which was simply synthesized in single step (See Figure 1 for structure). Selective turn-on emission and blue color enhancement of the **1**-RNA solution was observed which attributed to formation of stable complex of probe **1** with RNA, while it displays no particular response to other bioanions.[5] We also synthesised water soluble cyclic receptors **2** and **3** in single step[6,7] and these receptors also have similar key small naphthalene fluorophore which produce turn-on fluorescence on addition of RNA,

which is apparent from the blue color enhancement of the solution of **2** and **3** with RNA (Figure 2 and 3). These receptors produce weak fluorescence emission in phosphate buffer due to electron-deficient imidazolium moieties. The receptors **2** and **3** are isomers with different conformations and give turn-on fluorescence emission on addition of RNA with different intensities, however, at same wavelength of 450 nm. These two different intensities show that shape complementarity factor play an important role in developing interactions between receptors and RNA. The receptor **2** seems more fit in major groove of RNA and develop more strong π-π interactions of the aromatic part with nucleobases as compared to receptor **3** and give more fluorescence at 450 nm. Eventhough, this fluorescence emission is at low wavelength which could be increase in the future towards longer wavelength by introducing conjugations on naphthalene moiety. We used these probes (**2** and **3**) for RNA imaging in live cells. The probe **2** was used to target RNA in live onion cells and HeLa cell lines which produced bright blue fluorescence. Probe **2** was also applied in animal model system like Caenorhabditis elegans (C. elegans). **2** efficiently interacts with RNA and emits blue fluorescence from each cell of the whole embryo (Figure 2d ) which would help to investigate the RNA expression map of the embryo at different stages. While the probe **3** was used for bio-imaging of RNA in a neuronal cell line (SH-SY5Y cells) and green algae cells (*Chlamydomonas Reinhardtii*). In human neuroblastoma SH-SY5Y cell line, it was observed that the probe **3** was proficient of entering the cell and binding with the RNA in both cytosol and nucleus, giving rise to a strong blue fluorescence. However, after incubation with **3** in green algae cells, the cells also showed bright blue fluorescence in both cytoplasm and nucleus.

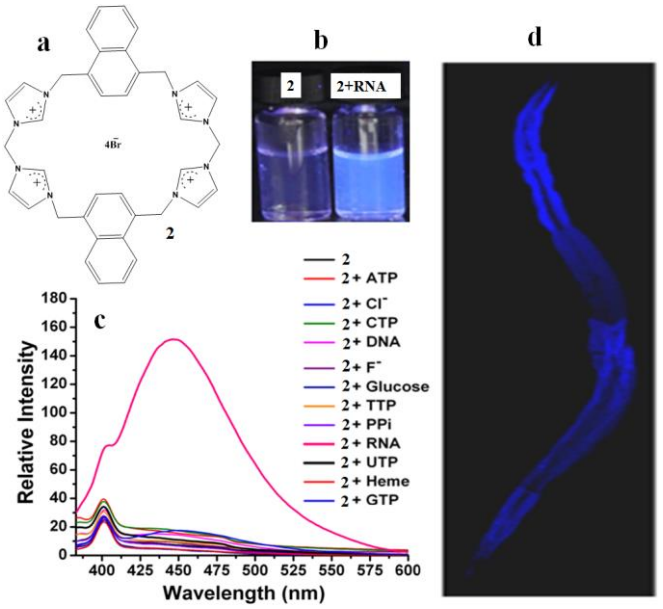



**Figure 2.** (a) Probe **2** (b) visual features of **2** and **2**-RNA (c) Fluorescence (slit width = 5 nm; excitation at 350nm) of probe **2** (10 μM) upon addition of guest molecules (1 equiv each) at pH 7.4 (10 mM phosphate buffer) (d) C. elegans stained with probe **2**. Reproduced with permission from American Chemical Society (ref. 7).

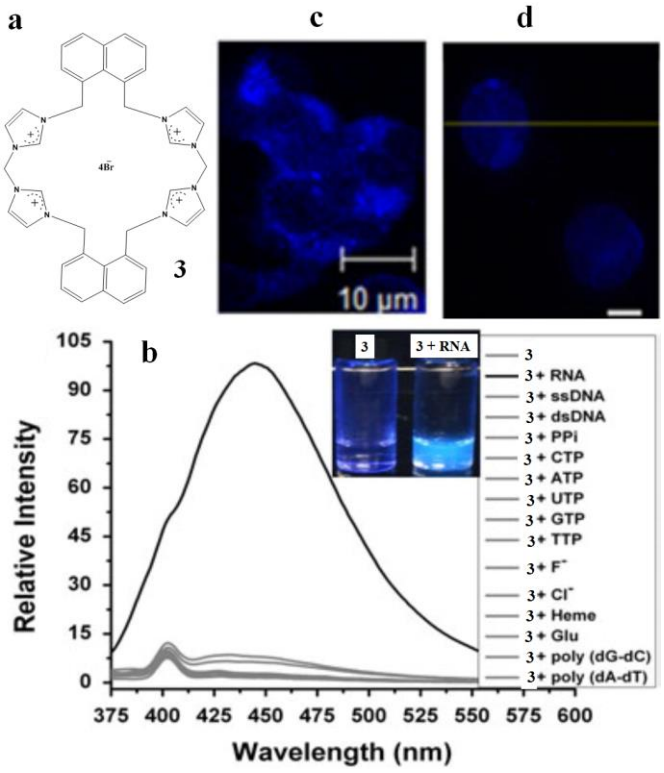



**Figure 3.** (a) Probe **3** (b) visual features of **3**, **3**-RNA and Fluorescence (slit width = 5 nm; excitation at 350nm) of probe **3** (10 μM) upon addition of guest molecules (1 equiv each) at pH 7.4 (10 mM phosphate buffer) (c) Fluorescence bright-field image of **3** in SH-SY5Y cells (d) SR-SIM images of green algae cells incubated with **3**. Reproduced with permission from Taylor & francis Ltd (ref. 8).

The RNA's major groove is the more electronegative potential area and results in turn-on selective detection of RNA while using probes **1**, **2**, and **3**, these probes have no significant response to other biomolecules. The primary factor for the increase in fluorescence emission in the presence of RNA is due to the strong electrostatic interaction of the charged probes (**1**, **2** and **3**) with the phosphate and hydroxyl groups of RNA which further help to generate π-stacking of the aromatic part of probes in the major groove region of RNA. This shape complementary factor of probes to the major groove

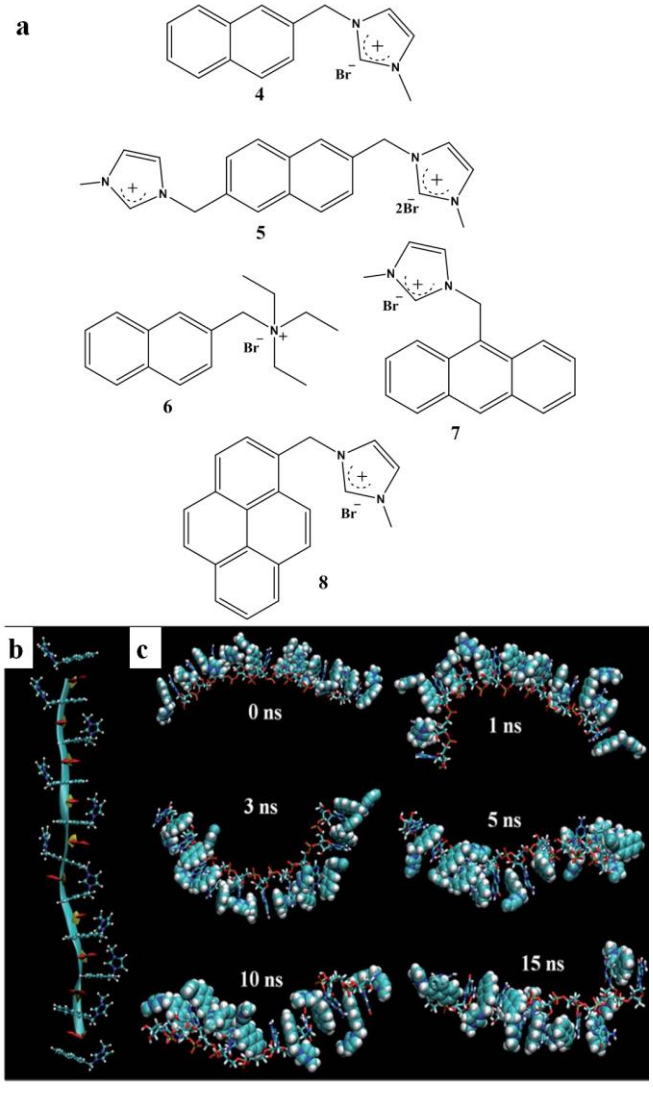



**Figure 4.** (a) Probes **4** – **8** (naphthalene based probes **4** – **6**, anthracene based probe **7** and pyrene based probe **8**) (b) MD simulation results; schematic description of tRNA fragment of 10 nucleotides interacting with probe **4**: naphthalene moieties at intercalation sites and imidazoium moieties interacting with phosphate backbone and 2′-hydroxyl group (2′-OH) of ribose (ribbon: phosphate backbone; ball-and-stick: probe 4; yellow: ribose; red: nucleobase). (c) Snapshots of 15 ns MD simulation in NPT ensemble of the corresponding model (stick: RNA; vdW: probe **4**). Water molecules are removed for clarity. Reproduced with permission from Royal Society of Chemistry (ref. 6).

of RNA play key role for selective binding and turn-on fluorescence. This binding mechanism was further strengthen by Kim research group.[6] They synthesized naphthalene-, anthracene- and pyrene-based probes. Interestingly, they observed that naphthalene-based probes show turn-on fluorescence with RNA moieties. Their theoretical calculations result showed that naphthalene-based charged probes fit and stacked strongly with nucleobase through π–π interactions as compared to other probes. This demonstrates that the shape complement factor of probes to the RNA moiety play an important role as well. Moreover, they performed molecular dynamics (MD) simulations for probe **4** with RNA containing 10 nucleotides. These MD simulations result indicate that naphthalene moieties have π–π interactions with nucleobases and imidazolium moieties interact with phosphate and ribose's 2′-OH besides these interactions form stable complex for 15 ns (See Figure 4) and this complexation time correspond to the time scale of fluorescence (1–100 ns) and rest of the other complexations of probe **4** with RNA were highly unstable. The MD simulation of the DNA fragment with the same RNA sequence form unstable complex for 0.4 ns due to lack of 2′-OH in DNA.

## Future directions

Targeting RNA with small molecules is a significant challenge. In the past few years, however, progress has been made to develop methods to design small molecules that bind to RNA with high affinity and specificity. Several novel synthetic small molecules have been identified to target RNA with good affinity and specificity. The facile synthetic approach to access fluorescent ionic probes and their solubility in aqueous media for selective recognition of RNA *in vitro* and *in vivo* studies, provide an easy way to access bio-sensors that rapidly response to the appearance of target RNA.[10] Many biological processes are controlled by RNA.[11] The selective detection and imaging of RNA molecules can provide information about their location, kinetics, and function at the cellular level,[12,13] allocate analysis of intracellular dynamics at a higher transitory resolution which can even track a single mRNA.[9b] The given positively charged probes (**1**-**6**) will help in further designing probes, for an in-depth investigation of RNAs and to see their therapeutic potential for drug discovery by using RNA expression data in the cells.[10] It is expected that continued advancements in live cell imaging of RNA will open new and exciting opportunities in a wide range of biological and medical applications. The effectiveness

and simplicity of these receptors suggest the potential development into a new methodology for practical real-time monitoring RNA transport in cells and spatial-temporal distribution of RNA in cellular functions.

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